FIGURE 1A

```
1    CGGCAGCAAAGGAACGTGCGAACGCGTGACGCCGCCCGACTGGCTCGCGCTCTCCCGTGC
61   CCCGGCGTCCTCCGCCCGCTCATGGCCCGGGCCGCCGCGGACGAGCGGCGCTGAGGCGGG
121  CCGCGTGGAGACGTGAGGCGGCCGCCGTGGCCCTCACAGTCGGCGTTTCGCCGCCTGCCC
181  GCGGTGCCCGCGCACGCCTGCCGCCATCGCCTTCGCGCCTGGCTGGCGGGGGCGCTGTCC
241  TCCCAGGCCGTCCGCGCCGCTCCCTGGAGCTCGGCGGAGCGCGGCAGCCAGGGCCGGCGG
301  AGGCGCGAGGAGCCGGGCGCCACCGCCGCCGCCGCCGCCGCCGCCGCGGGGGCCATGACC
361  GTGGAGCAGAACGTGCTGCAGCAGAGCGCGGCGCAGAAGCACCAGCAGACGTTTTTGAAT
421  CAACTGAGAGAAATTACGGGGATTAATGACACCCAGATACTACAGCAAGCCTTGAAGGAT
481  AGTAATGGAAACTTGGAATTAGCAGTGGCTTTCCTTACTGCGAAGAATGCTAAGACCCCT
541  CAGCAGGAGGAGACAACTTACTACCAAACAGCACTTCCTGGCAATGATAGATACATCAGT
601  GTGGGAAGCCAAGCAGATACAAATGTGATTGATCTCACTGGAGATGATAAAGATGATCTT
661  CAGAGAACAATTGCCTTGAGTTTGGCCGAATCAAACAGGGCATTCAGGGAGACTGGAATA
721  ACTGATGAGGAACAAGCCATTAGCAGAGTTCTTGAAGCCAGTATAGCAGAGAATAAAGCA
781  TGTTTGAAGAGGACACCTACAGAAGTTTGGAGGGATTCTCGAAACCCTTATGATAGAAAA
841  AGACAGGACAAAGCTCCCGTTGGGCTAAAGAATGTTGGCAATACTTGTTGGTTTAGTGCT
901  GTTATTCAGTCATTATTTAATCTTTTGGAATTTAGAAGATTAGTTCTGAATTACAAGCCT
961  CCATCAAATGCTCAAGATTTACCCCGAAACCAAAAGGAACATCGGAATTTGCCTTTTATG
1021 CGTGAGCTGAGGTATCTATTTGCACTTCTTGTTGGTACCAAAAGGAAGTATGTTGATCCA
1081 TCAAGAGCAGTTGAAATTCTTAAGGATGCTTTCAAATCAAATGACTCACAGCAGCAAGAT
1141 GTGAGTGAGTTTACACACAAATTATTAGATTGGTTAGAAGATGCCTTCCAAATGAAAGCT
1201 GAAGAGGAGACGGATGAAGAGAAGCCAAAGAACCCCATGGTAGAGTTGTTCTATGGCAGA
1261 TTCCTGGCTGTGGGAGTACTTGAAGGTAAAAAATTTGAAAACACTGAAATGTTTGGTCAG
1321 TACCCACTTCAGGTCAATGGGTTCAAAGATCTGCATGAGTGCCTAGAAGCTGCAATGATT
1381 GAAGGAGAAATTGAGTCTTTACATTCAGAGAATTCAGGAAAATCAGGCCAAGAGCATTGG
1441 TTTACTGGATTACCACCTGTGTTAACATTTGANTTGTCAAGATTTGAATTTAATCAGGCA
1501 TTGGGAAGACCAGAAAAAATTCACAACAAATTAGAATTTCCCCAAGTTTTATATTTGGAC
1561 AGATACATGCACAGAAACAGAGAAATAACAAGAATTAAGAGGGAAGAGATCAAGAGACTG
1621 AAAGATTACCTCACGGTATTACAACAAAGGCTAGAAAGATATTTAAGCTATGGTTCCGGT
1681 CCCAAACGATTCCCCTTGGTAGATGTTCTTCAGTATGCATTGGAATTTGCCTCAAGTAAA
1741 CCTGTTTGCACTTCTCCTGTTGACGATATTGACGCTAGTTCCCCACCTAGTGGTTCCATA
1801 CCATCACAGACATTACCAAGCACAACAGAACAACAGGGAGCCCTATCTTCAGAACTGCCA
1861 AGCACATCACCTTCATCAGTTGCTGCCATTTCATCGAGATCAGTAATACACAAACCATTT
1921 ACTCAGTCCCGGATACCTCCAGATTTGCCCATGCATCCGGCACCAAGGCACATAACGGAG
1981 GAAGAACTTTCTGTGCTGGAAAGTTGTTTACATCGCTGGAGGACAGAAATAGAAAATGAC
2041 ACCAGAGATTTGCAGGAAAGCATATCCAGAATCCATCGAACAATTGAATTAATGTACTCT
2101 GACAAATCTATGATACAAGTTCCTTATCGATTACATGCCGTTTTAGTTCACGAAGGCCAA
2161 GCTAATGCTGGGCACTACTGGGCATATATTTTTGATCATCGTGAAAGCAGATGGATGAAG
2221 TACAATGATATTGCTGTGACAAAATCATCATGGGAAGAGCTAGTGAGGGACTCTTTTGGT
2281 GGTTATAGAAATGCCAGTGCATACTGTTTAATGTACATAAATGATAAGGCACAGTTCCTA
2341 ATACAAGAGGAGTTTAATAAAGAAACTGGGCAGCCCCTTGTTGGTATAGAAACATTACCA
2401 CCGGATTTGAGAGATTTTGTTGAGGAAGACAACCAACGATTTGAAAAAGAACTAGAAGAA
2461 TGGGATGCACAACTTGCCCAGAAAGCTTTGCAGGAAAAGCTTTTAGCGTCTCAGAAATTG
2521 AGAGAGTCAGAGACTTCTGTGACAACAGCACAAGCAGCAGGAGACCCAGAATATCTAGAG
2581 CAGCCATCAAGAAGTGATTTCTCAAAGCACTTGAAAGAAGAAACTATTCAAATAATTACC
2641 AAGGCATCACATGAGCATGAAGATAAAAGTCCTGAAACAGTTTTGCAGTCGGCAATTAAG
2701 TTGGAATATGCAAGGTTGGTTAAGTTGGCCCAAGAAGACACCCCACCAGAAACCGATTAT
2761 CGTTTACATCATGTAGTGGTCTACTTTATCCAGAACCAGGCACCAAAGAAAATTATTGAG
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2881 ATAATGAAAGTTGCTCAAGCCAAACTGGAAATGATAAAACCTGAAGAAGTAAACTTGGAG
2941 GAATATGAGGAGTGGCATCAGGATTATAGGAAATTCAGGGAAACAACTATGTATCTCATA
3001 ATTGGGCTAGAAAATTTTCAAAGAGAAAGTTATATAGATTCCTTGCTGTTCCTCATCTGT
3061 GCTTATCAGAATAACAAAGAACTCTTGTCTAAAGGCTTATACAGAGGACATGATGAAGAA
3121 TTGATATCACATTATAGAAGAGAATGTTTGCTAAAATTAAATGAGCAAGCCGCAGAACTC
3181 TTCGAATCTGGAGAGGATCGAGAAGTAAACAATGGTTTGATTATCATGAATGAGTTTATT
3241 GTCCCATTTTTGCCATTATTACTGGTGGATGAAATGGAAGAAAAGGATATACTAGCTGTA
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3481 ATCATGTTGTCCCTCAGTCGAACTCCTGCTGATGGAAGA**TAA**ACTGCACACTTTCCCTGA
3541 ACACACTGTATAAACTCTTTTAGTTCTTAACCCTTGCCTTCCTGTCACAGGGTTTGCTT
3601 GTTGCTGCTATAGTTTTTAACTTTTTTTATTTTAATAACTGCAAAAGACAAAATGACTA
3661 TACAGACTTTAGTCAGACTGCAGACAATAAAGCTGAAAATCGCATGGCGCTCAGACATTT
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3841 ATTCAGTCTGGATCCTTCCATGACTACAGCCATTTAAGTGTTCAGCACTGTGTACGATAC
3901 ATAATATTTGGTAGCTTGTAAATGAAATAAAGAATAAAGTTTTATTTATGGCTAC

FIGURE 1B

FIGURE 2

```
1    MTVEQNVLQQSAAQKHQQTFLNQLREITGINDTQILQQALKDSNGNLELAVAFLTAKNAK
61   TPQQEETTYYQTALPGNDRYISVGSQADTNVIDLTGDDKDDLQRTIALSLAESNRAFRET
121  GITDEEQAISRVLEASIAENKACLKRTPTEVWRDSRNPYDRKRQDKAPVGLKNVGNTCWF
181  SAVIQSLFNLLEFRRLVLNYKPPSNAQDLPRNQKEHRNLPFMRELRYLFALLVGTKRKYV
241  DPSRAVEILKDAFKSNDSQQQDVSEFTHKLLDWLEDAFQMKAEEETDEEKPKNPMVELFY
301  GRFLAVGVLEGKKFENTEMFGQYPLQVNGFKDLHECLEAAMIEGEIESLHSENSGKSGQE
361  HWFTGLPPVLTFXLSRFEFNQALGRPEKIHNKLEFPQVLYLDRYMHRNREITRIKREEIK
421  RLKDYLTVLQQRLERYLSYGSGPKRFPLVDVLQYALEFASSKPVCTSPVDDIDASSPPSG
481  SIPSQTLPSTTEQQGALSSELPSTSPSSVAAISSRSVIHKPFTQSRIPPDLPMHPAPRHI
541  TEEELSVLESCLHRWRTEIENDTRDLQESISRIHRTIELMYSDKSMIQVPYRLHAVLVHE
601  GQANAGHYWAYIFDHRESRWMKYNDIAVTKSSWEELVRDSFGGYRNASAYCLMYINDKAQ
661  FLIQEEFNKETGQPLVGIETLPPDLRDFVEEDNQRFEKELEEWDAQLAQKALQEKLLASQ
721  KLRESETSVTTAQAAGDPEYLEQPSRSDFSKHLKEETIQIITKASHEHEDKSPETVLQSA
781  IKLEYARLVKLAQEDTPPETDYRLHHVVVYFIQNQAPKKIIEKTLLEQFGDRNLSFDERC
841  HNIMKVAQAKLEMIKPEEVNLEEYEEWHQDYRKFRETTMYLIIGLENFQRESYIDSLLFL
901  ICAYQNNKELLSKGLYRGHDEELISHYRRECLLKLNEQAAELFESGEDREVNNGLIIMNE
961  FIVPFLPLLLVDEMEEKDILAVEDMRNRWCSYLGQEMEPHLQEKLTDFLPKLLDCSMEIK
1021 SFHEPPKLPSYSTHELCERFARIMLSLSRTPADGR
```

FIGURE 3A

```
1    CGGCAGCAAAGGAACGTGCGAACGCGTGACGCCGCCCGACTGGCTCGCGCTCTCCCGTGC
61   CCCGGCGTCCTCCGCCCGCTCATGGCCCGGGCCGCCGCGGACGAGCGGCGCTGAGGCGGG
121  CCGCGTGGAGACGTGAGGCGGCCGCCGTGGCCCTCACAGTCGGCGTTTCGCCGCCTGCCC
181  GCGGTGCCCGCGCACGCCTGCCGCCATCGCCTTCGCGCCTGGCTGGCGGGGGCGCTGTCC
241  TCCCAGGCCGTCCGCGCCGCTCCCTGGAGCTCGGCGGAGCGCGGCAGCCAGGGCCGGCGG
301  AGGCGCGAGGAGCCGGGCGCCACCGCCGCCGCCGCCGCCGCCGCCGCGGGGGCCATGACC
361  GTGGAGCAGAACGTGCTGCAGCAGAGCGCGGCGCAGAAGCACCAGCAGACGTTTTTGAAT
421  CAACTGAGAGAAATTACGGGGATTAATGACACCCAGATACTACAGCAAGCCTTGAAGGAT
481  AGTAATGGAAACTTGGAATTAGCAGTGGCTTTCCTTACTGCGAAGAATGCTAAGACCCCT
541  CAGCAGGAGGAGACAACTTACTACCAAACAGCACTTCCTGGCAATGATAGATACATCAGT
601  GTGGGAAGCCAAGCAGATACAAATGTGATTGATCTCACTGGAGATGATAAAGATGATCTT
661  CAGAGAACAATTGCCTTGAGTTTGGCCGAATCAAACAGGGCATTCAGGGAGACTGGAATA
721  ACTGATGAGGAACAAGCCATTAGCAGAGTTCTTGAAGCCAGTATAGCAGAGAATAAAGCA
781  TGTTTGAAGAGGACACCTACAGAAGTTTGGAGGGATTCTCGAAACCCTTATGATAGAAAA
841  AGACAGGACAAAGCTCCCGTTGGGCTAAAGAATGTTGGCAATACTTGTTGGTTTAGTGCT
901  GTTATTCAGTCATTATTTAATCTTTTGGAATTTAGAAGATTAGTTCTGAATTACAAGCCT
961  CCATCAAATGCTCAAGATTTACCCCGAAACCAAAAGGAACATCGGAATTTGCCTTTTATG
1021 CGTGAGCTGAGGTATCTATTTGCACTTCTTGTTGGTACCAAAAGGAAGTATGTTGATCCA
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1921 ACTCAGTCCCGGATACCTCCAGATTTGCCCATGCATCCGGCACCAAGGCACATAACGGAG
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3721 TAACCGGAACTGATGTATAATCACAAATCTAATTGATTTTATTATGGCAAAACTATGCTT
3781 TTGCCACCTTCCTGTTGCAGTATTACTTTGCTTTTATCTTTTCTTTCTCAACAGCTTTCC
3841 ATTCAGTCTGGATCCTTCCATGACTACAGCCATTTAAGTGTTCAGCACTGTGTACGATAC
3901 ATAATATTTGGTAGCTTGTAAATGAAATAAAGAATAAAGTTTTATTTATGGCTAC
```

FIGURE 3B

FIGURE 4

```
1    MTVEQNVLQQSAAQKHQQTFLNQLREITGINDTQILQQALKDSNGNLELAVAFLTAKNAK
61   TPQQEETTYYQTALPGNDRYISVGSQADTNVIDLTGDDKDDLQRTIALSLAESNRAFRET
121  GITDEEQAISRVLEASIAENKACLKRTPTEVWRDSRNPYDRKRQDKAPVGLKNVGNTCWF
181  SAVIQSLFNLLEFRRLVLNYKPPSNAQDLPRNQKEHRNLPFMRELRYLFALLVGTKRKYV
241  DPSRAVEILKDAFKSNDSQQQDVSEFTHKLLDWLEDAFQMKAEEETDEEKPKNPMVELFY
301  GRFLAVGVLEGKKFENTEMFGQYPLQVNGFKDLHECLEAAMIEGEIESLHSENSGKSGQE
361  HWFTGLPPVLTFXLSRFEFNQALGRPEKIHNKLEFPQVLYLDRYMHRNREITRIKREEIK
421  RLKDYLTVLQQRLERYLSYGSGPKRFPLVDVLQYALEFASSKPVCTSPVDDIDASSPPSG
481  SIPSQTLPSTTEQQGALSSELPSTSPSSVAAISSRSVIHKPFTQSRIPPDLPMHPAPRHI
541  TEEELSVLESCLHRWRTEIENDTRDLQESISRIHRTIELMYSDKSMIQVPYRLHAVLVHE
601  GQANAGHYWAYIFDHRESRWMKYNDIAVTKSSWEELVRDSFGGYRNASAYCLMYINDKAQ
661  FLIQEEFNKETGQPLVGIETLPPDLRDFVEEDNQRFEKELEEWDAQLAQKALQEKLLASQ
721  KLRESETSVTTAQAAGDPEYLEQPSRSDFSKHLKEETIQIITKASHEHEDKSPETVLQSA
781  IKLEYARLVKLAQEDTPPETDYRLHHVVVYFIQNQAPKKIIEKTLLEQFGDRNLSFDERC
841  HNIMKVAQAKLEMIKPEEVNLEEYEEWHQDYRKFRETTMYLIIGLENFQRESYIDSLLFL
901  ICAYQNNKELLSKGLYRGHDEELISHYRRECLLILNLKRKQKPILFFFLHCIKKLNEQAA
961  ELFESGEDREVNNGLIIMNEFIVPFLPLLLVDEMEEKDILAVEDMRNRWCSYLGQEMEPH
1021 LQEKLTDFLPKLLDCSMEIKSFHEPPKLPSYSTHELCERFARIMLSLSRTPADGR
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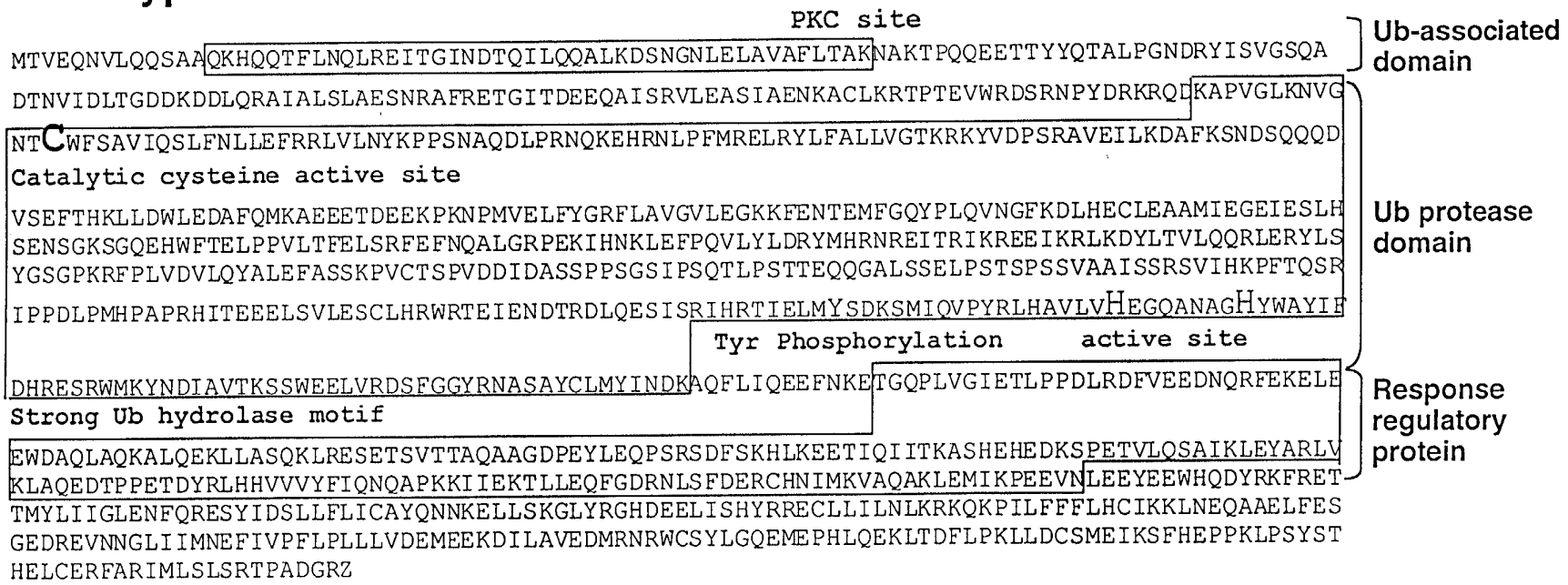
Figure 5

Sequence of SUP

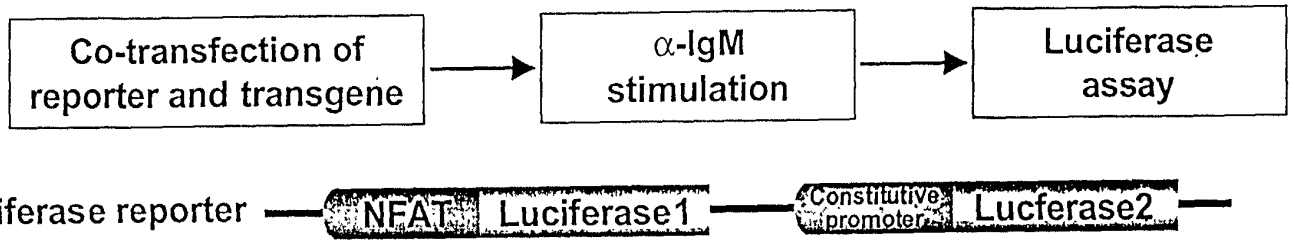
Wild type SUP



Mutant SUP

Cys → Ser

mtSUP Suppresses α-IgM Induced NFAT-Luciferase Activity as a Dominant-negative Mutant



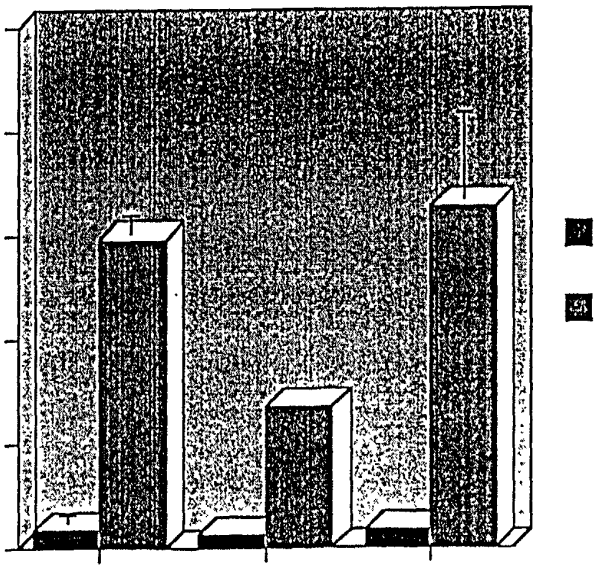
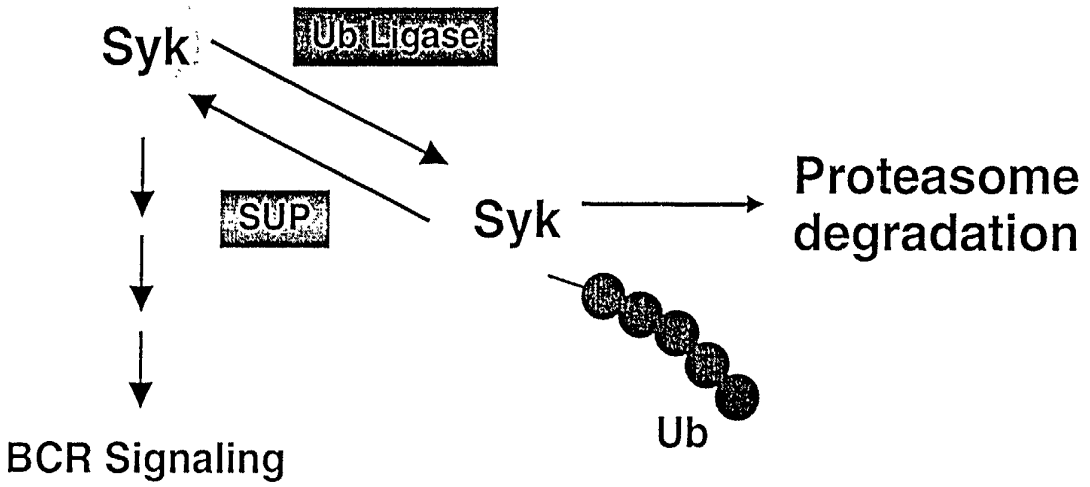
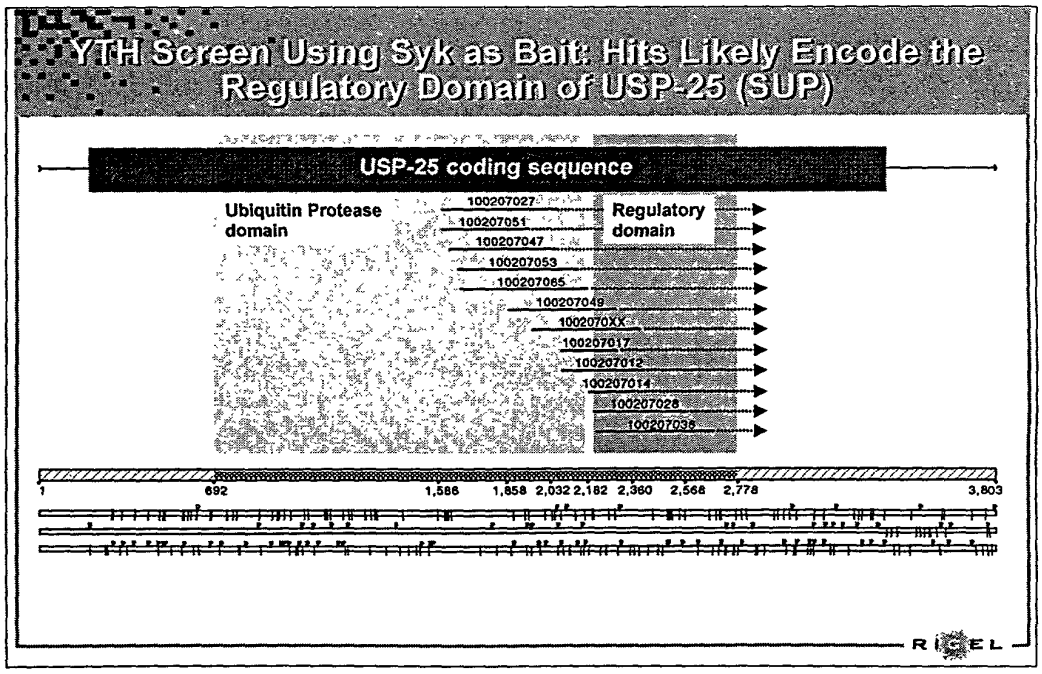


Figure 7

Model: SUP Regulates BCR Signaling by Stabilizing Syk

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Ubiquitin Protease domain
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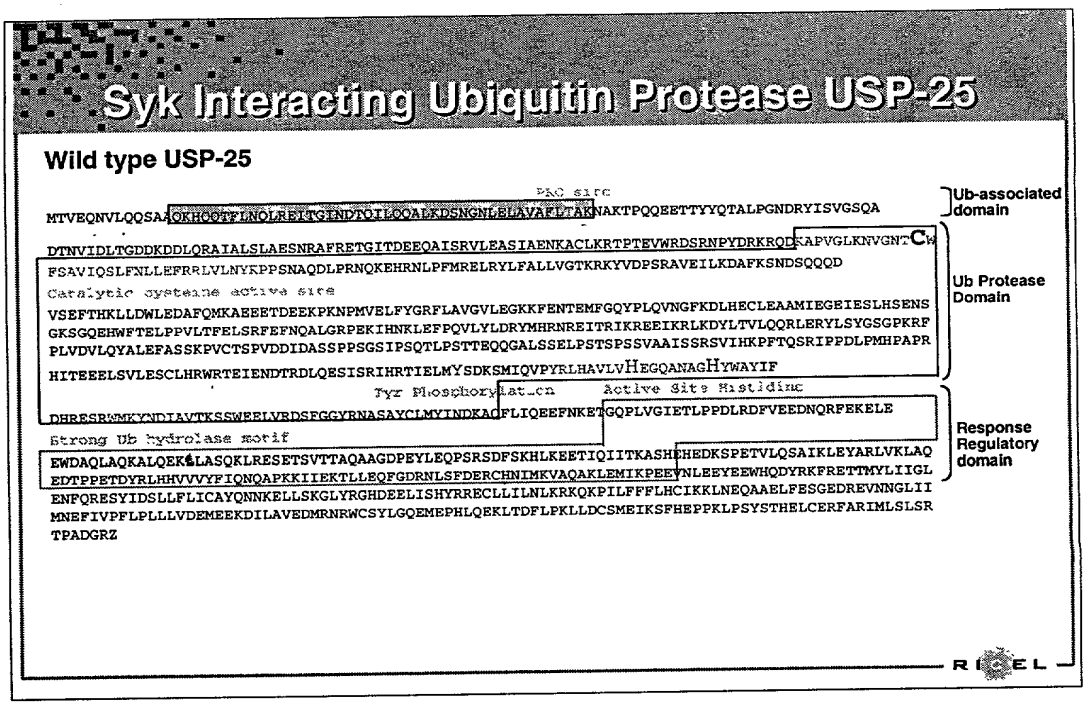
3,803
RIGEL

Fig. 8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Tyr Phosphorylation
Active Site Histidine
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Strong Ub hydrolase motif
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EDTPPETDYRLHHVVVYFIQNQAPKKIIEKTLLEQFGDRNLSFDERCHNIMKVAQAKLEMIKPEEVNLEEYEEWHQDYRKFRETTMYLIIGL
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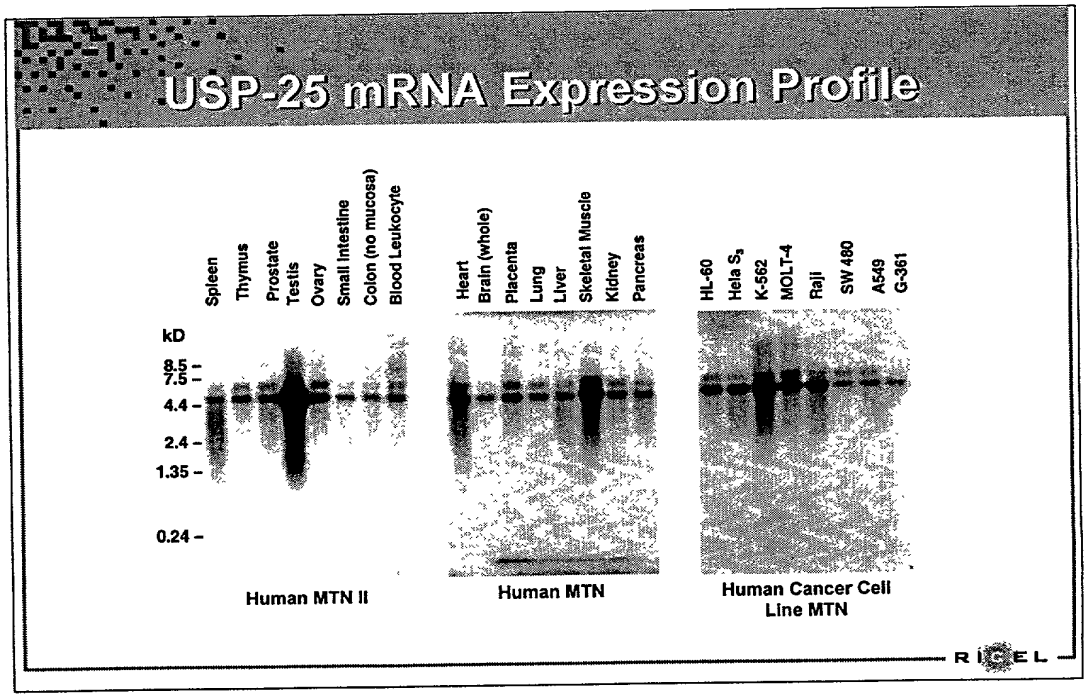
TPADGRZ
Ub-associated domain
Ub Protease Domain
Response Regulatory domain
RIGEL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Thymus
Prostate
Testis
Ovary
Small Intestine
Colon (no mucosa)
Blood Leukocyte
Heart
Brain (whole)
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
HL-60
Hela S3
K-562
MOLT-4
Raji
SW 480
A549
G-361
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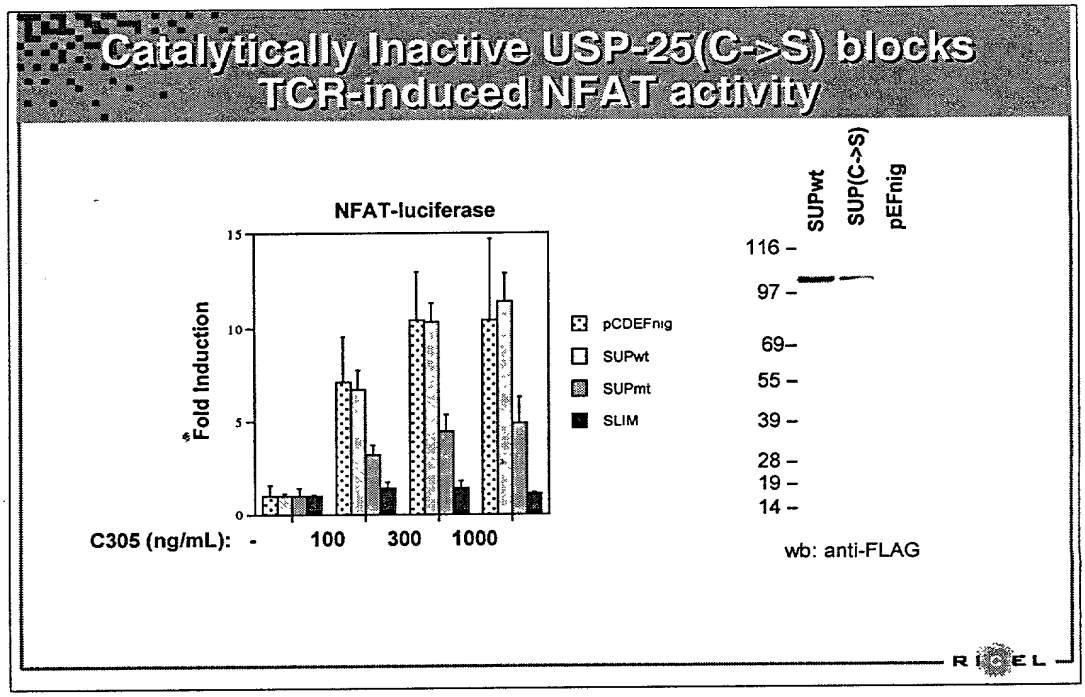
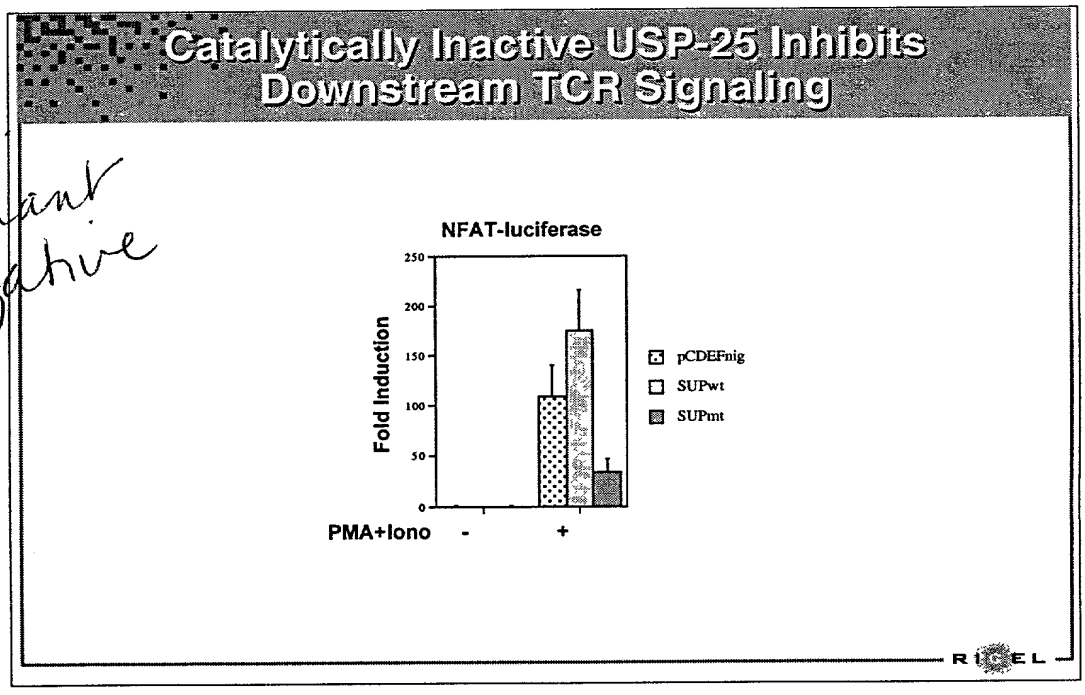
0.24
Human MTN II
Human MTN
Human Cancer Cell Line MTN
RIGEL

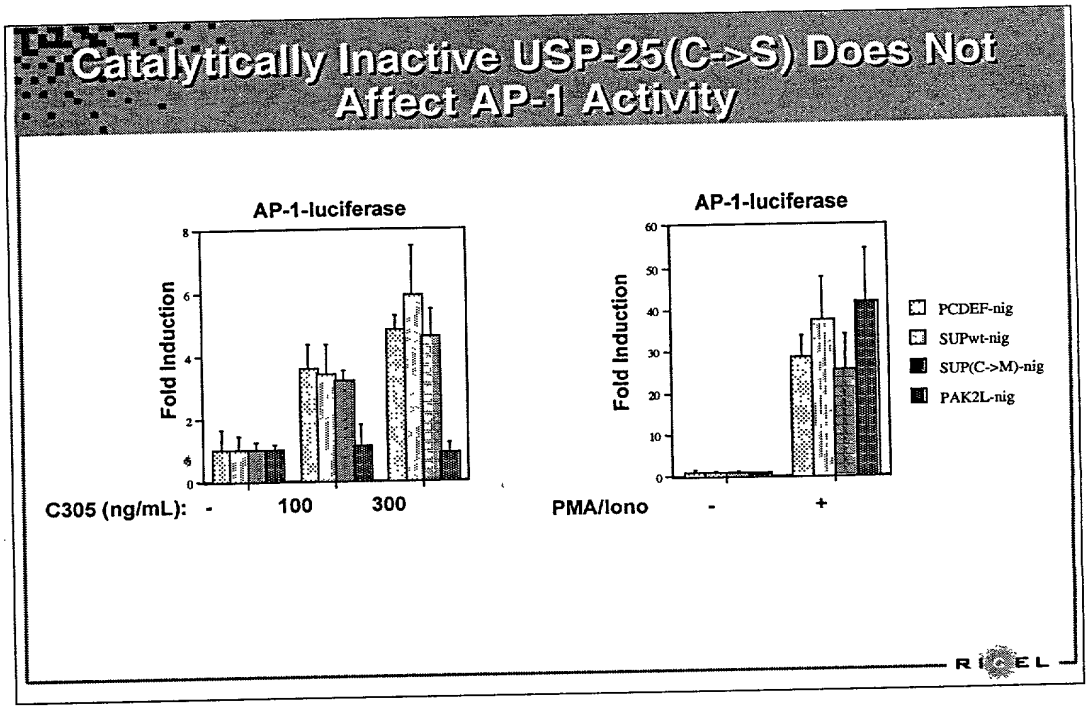
Fig. 9





Dominant negative

Fig. 10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SUPwt-nig
SUP(C->M)-nig
PAK2L-nig
RIGEL

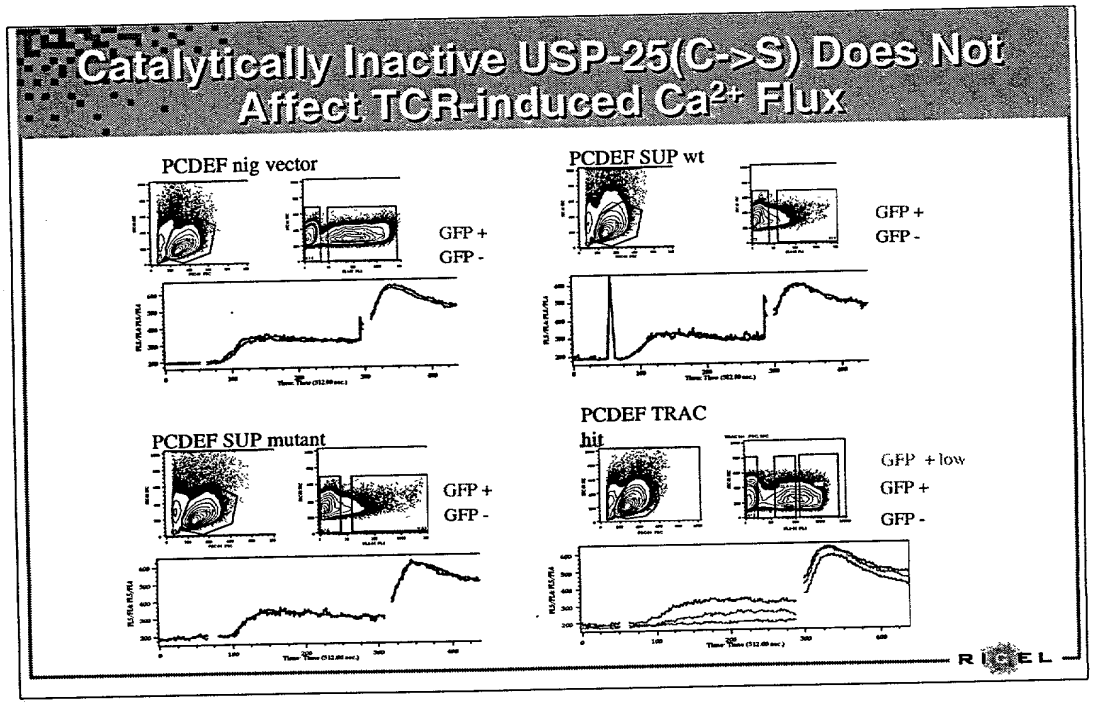
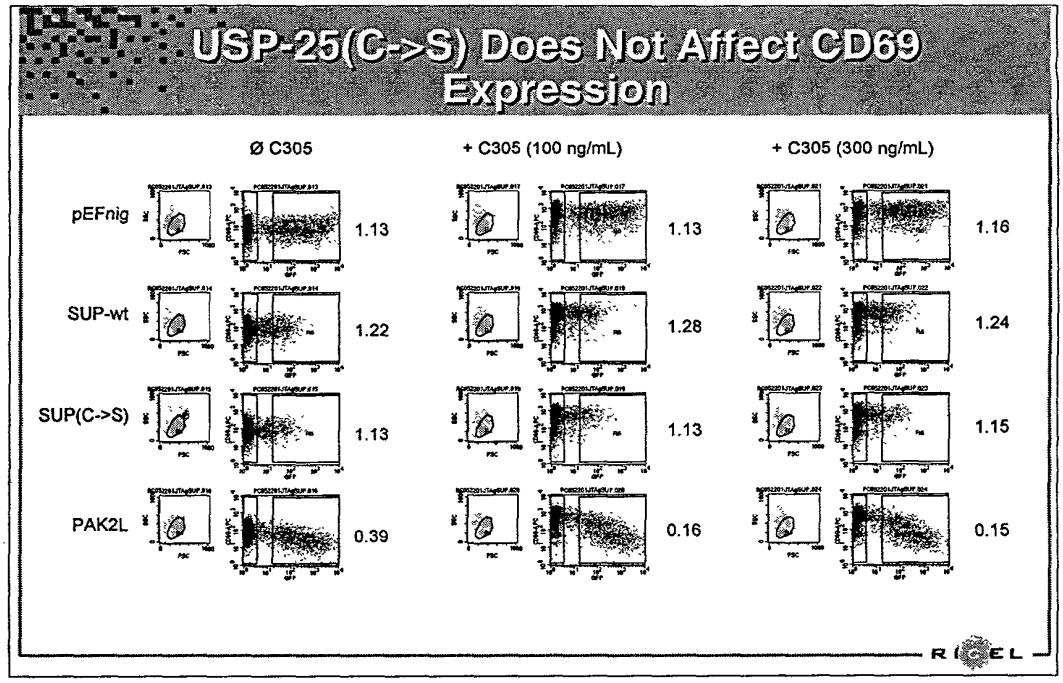

Catalytically Inactive USP-25(C->S) Does Not Affect TCR-induced Ca2+ Flux
PCDEF nig vector
GFP +
GFP -
PCDEF SUP wt
GFP +
GFP -
PCDEF SUP mutant
GFP +
GFP -
PCDEF TRAC hit
GFP +low
GFP +
GFP -
RIGEL

Fig. 11


USP-25(C->S) Does Not Affect CD69 Expression
Ø C305
+ C305 (100 ng/mL)
+ C305 (300 ng/mL)
pEFnig
1.13
1.13
1.16
SUP-wt
1.22
1.28
1.24
SUP(C->S)
1.13
1.13
1.15
PAK2L
0.39
0.16
0.15
RIGEL

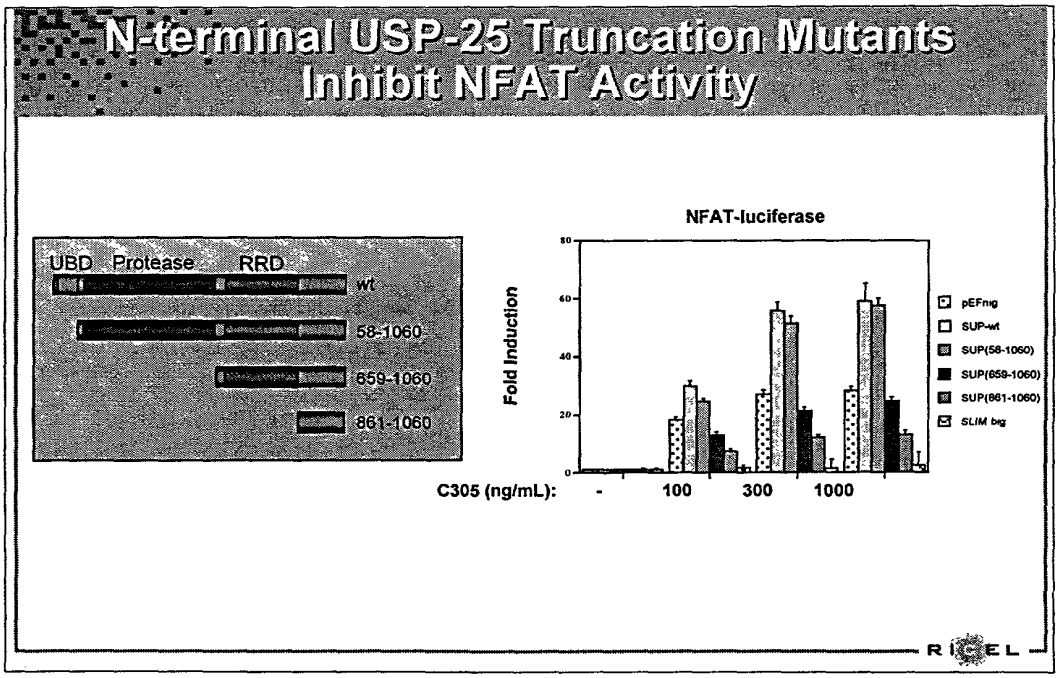
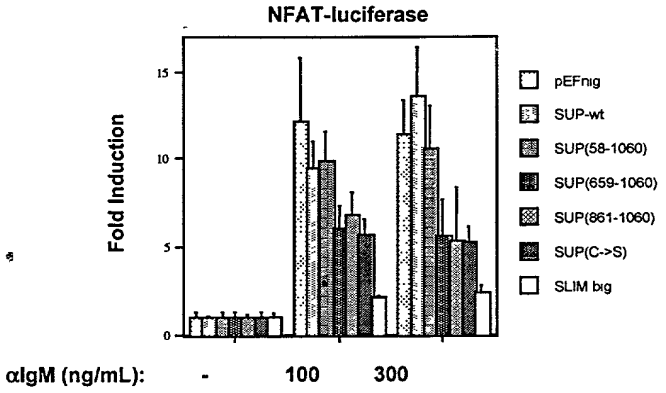

N-terminal USP-25 Truncation Mutants Inhibit NFAT Activity
UBD
Protease
RRD
wt
58-1060
659-1060
861-1060
NFAT-luciferase
Fold Induction
C305 (ng/mL):
-
100
300
1000
pEFnig
SUP-wt
SUP(58-1060)
SUP(659-1060)
SUP(861-1060)
SLIM big
RIGEL

Fig. 12

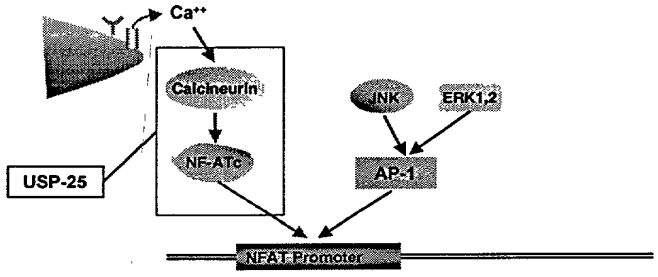
USP-25 Mutants Inhibit NFAT activity in BJABs



RICEL

Possible Regulatory Role of USP-25 on NFAT Activity

- USP-25 likely regulates the NFAT promoter downstream of Ca^{2+} and independent of the AP-1 pathway



RICEL

Fig. 13

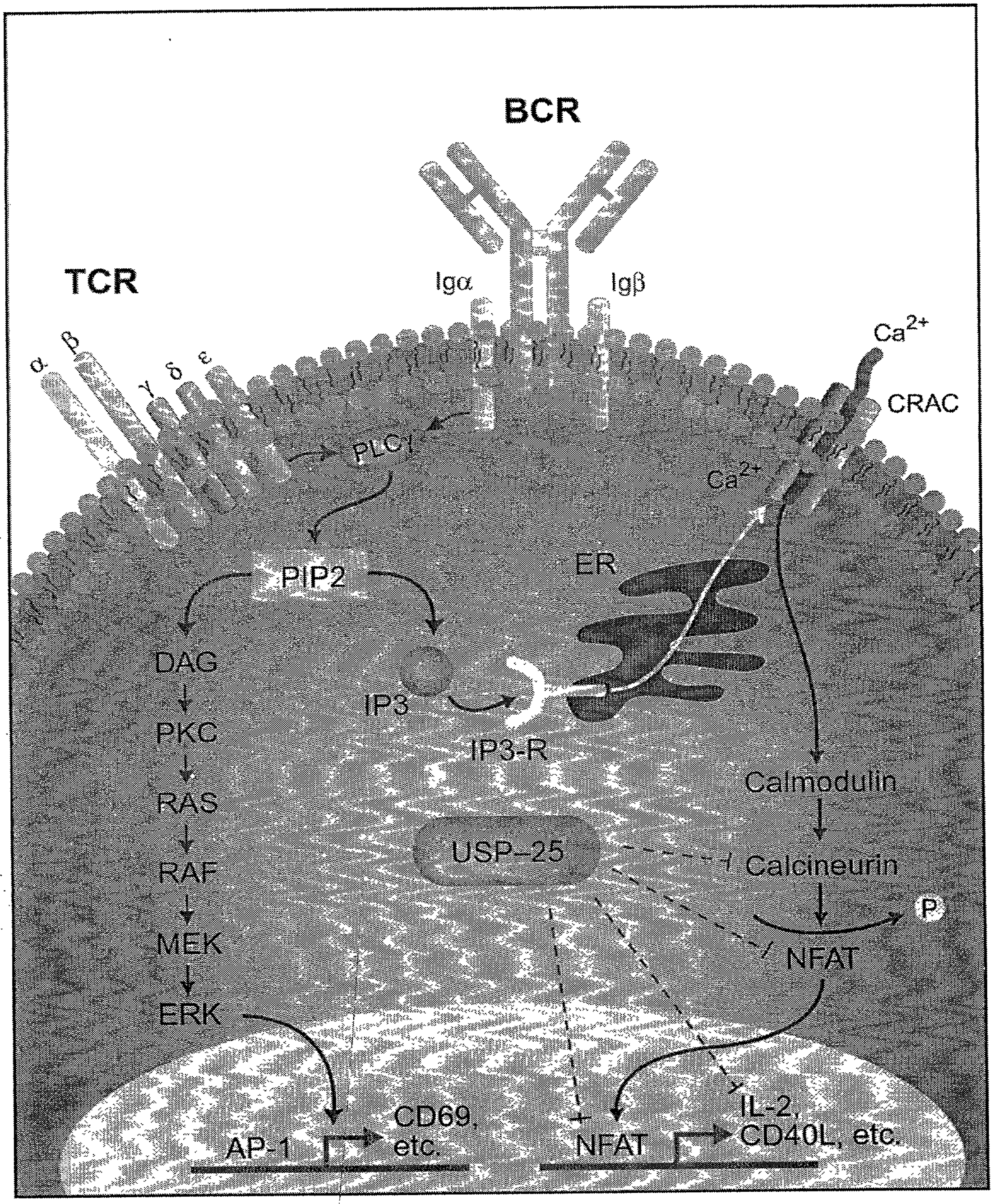

BCR
TCR
Igα
Igβ
α
β
γ
δ
ε
Ca2+
CRAC
PLCγ
Ca2+
PIP2
ER
DAG
PKC
RAS
RAF
MEK
ERK
IP3
IP3-R
Calmodulin
USP-25
Calcineurin
P
NFAT
AP-1
CD69,
etc.
NFAT
IL-2,
CD40L, etc.

Fig. 14